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UTILIZATION OF SLAUGHTER-HOUSE BYPRODUCTS AS NITROGEN SOURCE FOR FILAMENTOUS FUNGI IN SUBMERGED FERMENTATION, WITH SIMULTANEOUS DEODORIZATION

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UTILIZACION DE LOS SUBPRODUCTOS DEL RASTRO COMO FUENTE DE NITROGENO PARA EL CRECIMIENTO DE HONGOS FILAMENTOSOS EN CULTIVO SUMERGIDO, CON EFECTO DESODORANTE SIMULTANEO

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SUMMARY

The potential of slaughter-house byproducts (the meals of feathers, hairs, blood and meat scraps) as sole source of nitrogen in sustaining growth of *Trichoderma harzianum* and *Aspergillus niger* in submerged fermentation was studied. The effect of different C/N ratios and the substitution of glucose with other polymeric cheaper carbon sources, such as cassava starch, showed that the efficacy of individual meals as sole source of nitrogen varies depending on the type of meal and microbial species. The data established that these abundantly available slaughter-house

byproducts can be used as low cost nitrogen sources for gaining economic advantages in fungal submerged fermentations. Simultaneous complete deodorization of the meals was observed at the end of fermentation in most cases, which was not due to complete utilization of the meal present in the medium. This opens up an entirely new approach for microbial deodorization of these meals, which will make them more acceptable as components of animal feed, due to elimination of offensive odours, and may also provide higher returns than what is possible at present.

Key words: Filamentous fungi, nitrogen source, fermentation, feathers, hairs, blood, meat scraps, *Trichoderma harzianum*, *Aspergillus niger*, deodorization.

RESUMEN

La producción extensiva de carne para consumo humano implica la obtención de un volumen importante de subproductos con alto contenido de proteínas. Dos factores limitan su utilización en la formulación de alimentos para ganado: 1) olor desagradable, y 2) desequilibrio en la composición de aminoácidos, lo cual disminuye su valor nutritivo. Se evaluaron cuatro de estos subproductos (harinas de: plumas, pelos, sangre y carne) como substrato para el crecimiento en medio líquido de dos hongos filamentosos: *Trichoderma harzianum* y *Aspergillus niger*. Se estudiaron diferentes valores de la relación C/N, utilizando como fuente de carbono glucosa o harina de yuca. Ambas especies fueron capaces de utilizar los diferentes subproductos como fuente única de nitrógeno. Sin embargo, la eficiencia de cada una de las harinas como substrato depende del microorganismo utilizado. En todos los casos, se obtuvo una desodorización completa de los medios de cultivo después de 25 horas de fermentación, incluso antes del consumo completo de las harinas. Este trabajo abre nuevas perspectivas sobre la aplicación de un nuevo proceso para eliminar los malos olores de las harinas de los subproductos del rastro, así como para la producción de diferentes metabolitos de hongos filamentosos con alto valor agregado.

Palabras clave: Hongos filamentosos, fuente de nitrógeno, fermentación, *Trichoderma harzianum, Aspergillus niger*, subproductos del rastro, harinas de plumas, pelos, sangre y carne.

INTRODUCTION

Increased world population and higher demands for animal products in the recent years have given impetus to animal husbandry, as well as animal product processing industries throughout the world. These developments, in

turn, have culminated into generation of different kinds of animal byproducts in quantities. These byproducts, although extremely rich in organic matter, can cause environmental pollution if they are not properly treated before discharge into natural streams. This special treatment, however, involves high capital and operating expenses, without providing any financial returns to the industry in most cases (Lonsane and Krishnaiah, 1991). Consequently, the utilization of the byproducts, to obviate the need for waste treatment, is preferred (Lonsane and Ahmed, 1989).

A number of industries have been established in Latin American countries, especially in Mexico, for processing slaughter-house byproducts, such as hairs, feathers, blood and meat scraps into meals (Ensiminger, 1978; Conrad and Campabadal, 1979). The only avenue available at present for utilization of these meals is as a component of animal feed. However, they fetch very low prices due to different limitations, such as lower digestibility, poor protein quality, presence of toxic factors and highly offensive odours. It is of vital importance to these countries to find additional uses for these meals in order to get better returns to the industry and a more efficient utilization.

The present study reports the potential of four different slaughter-house byproducts (the meals of hairs, feathers, blood and meat scraps) as nitrogen source in fungal submerged fermentations and their simultaneous deodorization.

MATERIALS AND METHODS

Microorganisms

Trichoderma harzianum Rifai, strain CCM F-470, was obtained from the Czechoslovak Collection of Microorganisms, Brno, Czechoslovakia (Roussos, 1987); while *Aspergillus niger* van Tieghem, strain ORSTOM No. 10, was obtained from the ORSTOM strain collection, Montpellier, France (Raimbault, 1980). Both strains were maintained and subcultured on potato dextrose agar at 4°C. For inoculum preparation, the spores from freshly grown slants were suspended in 0.01% solution of Tween 80 (10 ml/slant). The inoculum ratio was 3×10^7 spores/g carbon source initially present in the culture medium.

Submerged fermentation
The basal medium (pH 5.6) contained (g/L distilled water): KH_2PO_4 1.3, Na_2HPO_4 0.12, $MgSO_4.7H_2O$ 0.3, $CaCl_2.2H_2O$ 0.3, vitamin-mineral salt solution 2 (Roussos, 1982). Glucose at 0.2 or 1.0% levels as carbon source and individual meals (moisture content 8-10%; obtained from "Energéticos para Alimentos Balanceados, S. A. de C. V.", Iztapaluca, Mexico), as sole nitrogen source and to give 1, 7, 10, 13, 16 and 19 ratios of C/N, were added prior to sterilization. 50 ml of the medium were sterilized in 250 ml capacity Erlenmeyer flasks at 115°C for 30 min. The inoculated flasks were incubated on rotary shaker (100 rpm) for 70 h, at 29° and 35°C for the experiments of *T. harzianum* and *A. niger*, respectively. In another set of experiments, cassava meal and wheat bran were used individually in place of glucose at a C/N ratio of 10. The growth kinetics of the cultures in Biolaffite fermenter (1.5 L working capacity), with automatic controls, were also studied with glucose as carbon source at 0.2 and 1.0% levels and a C/N ratio of 10 in both cases. The fermentation medium was agitated at 200 rpm, while the air supply was at the rate of 60 L/h and the temperature was regulated at 29° and 35°C.

Analytical aspects
The samples were homogenized for 1 min in an Ultra-turrax (Janke & Kunkel, Germany) at 20 000 rotations per min before centrifugation at 6000 rpm for 15 min. The supernatant was used in estimation of reducing sugars by the DNS method (Miller, 1959), and starch as well as cellulose by the Anthrone method (Dubois *et al.*, 1956), using glucose as standard sugar.

The malodour of the meals at the end of a 70 h fermentation period was determined qualitatively by a panel of 5 researchers. The absence of the characteristic malodour of the meals was taken as a criterion of complete deodorization. The characteristic mycelial odour of the culture was, however, ignored while determining the deodorization.

RESULTS AND DISCUSSION

Utilization of meals as nitrogen source
The growth of the microorganism in the medium is the most appropriate criterion of its ability to use the meal, because the basal medium employed was devoid of any other nitrogen source. Direct biomass determination was not possible due to difficulties in separating the microbial cells from the meal.

The mycelial cells of *Aspergillus niger* formed pellets and enveloped all the individual particles of the meals and, consequently, the medium became almost clear by 48 h. However, no pellets were formed by *Trichoderma harzianum*. Similarly, the estimation of protein as growth indicator was not possible due to the problems in distinguishing mycelial protein from substrate protein in the fermentation media. Therefore, the growth of the culture in the present studies was determined indirectly based on the consumption of the carbon substrate. The data indicated that both cultures were able to efficiently use each of the all four meals individually as sole source of nitrogen (C/N ratio of 1), as the consumption of glucose was up to 94% in almost all the cases (Table 1); the sole exception being the consumption of 77.5% glucose by *A. niger* in the medium containing blood meal. However, the ability varies depending on the type of the meal and also on the microbial species.

The meals of the slaughter-house byproducts can be used efficiently as sole source of nitrogen in submerged fermentations involving *A. niger* and *T. harzianum*. These results may also be applicable to other fungal cultures, yeasts and bacteria, as well as a solid state fermentation (SSF) system. These meals are available in abundance in Mexico and other Latin American countries, and are being used at present as a component of animal feed, in spite of their limitations.

Effect of C/N ratios on glucose consumption

The consumption of glucose by *A. niger* increased with the increase in C/N ratios in the media containing meat and blood meals, while it was decreased

Table 1. Utilization of the meals as nitrogen source at a C/N ratio of 1 by *Trichoderma harzianum* and *Aspergillus niger*.

Nitrogen source	% consumption of glucose at 70 h	
	T. harzianum	*A. niger*
Blood meal	94.1	77.5
Feather meal	94.4	95.8
Hair meal	100.0	100.0
Meat meal	94.4	94.1

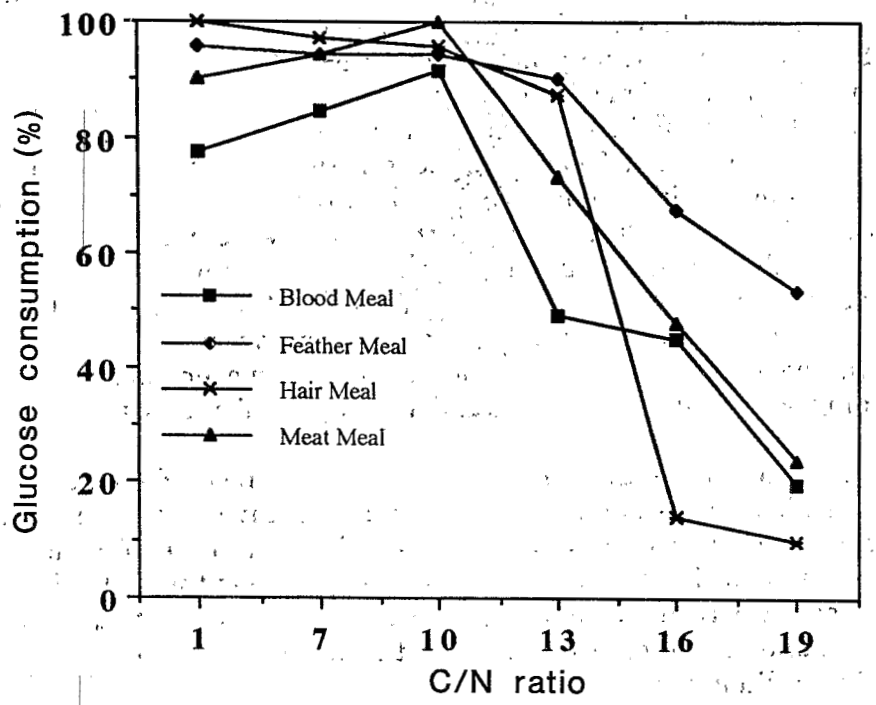


Fig. 1. Effect of C/N ratios on consumption of glucose by *Aspergillus niger*. Temperature 35°C, time= 70 h.

slightly between C/N ratios of 1-10 in media containing hair and feather meals (Fig 1). The total glucose consumption was up to 90% for all the meals with C/N ratios up to 13, excepting the media containing blood and meat meals. On the other hand, the consumption of glucose by *T. harzianum* was up to 90% at the C/N ratios up to 13, excepting the hair and meat meals (Fig. 2). It increased with the increase in the C/N ratios up to 13 in blood meal containing medium, and remained at 100% even when the ratio was 19. In all other media, the glucose consumption by both fungal cultures was found to decrease according to the increase in C/N ratios beyond 10. This is probably due to limitations of the nitrogen source. The final pH in case of *T. harzianum* was 5.2-5.4 in the media containing different meals, in contrast to its more acidic range (4.5-4.9) in case of *A. niger*.

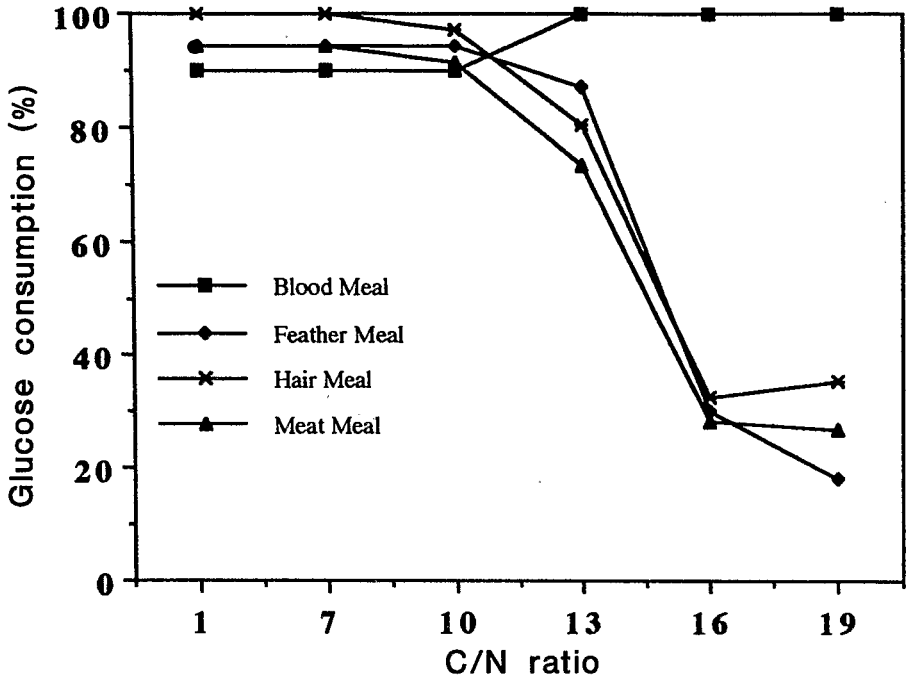


Fig. 2. Glucose consumption by *Trichoderma harzianum* at different C/N ratios. Temperature 29°C, time 70= h.

Kinetic studies

The kinetics of glucose consumption in a 1.5 L fermenter by *A. niger* and *T. harzianum* are shown in figures 3-4, respectively. The glucose consumption (%) was found to increase with time in both cultures. The lower consumption of glucose in the media containing 10 g initial glucose/L is probably due to the limitation of nitrogen, because the medium contained only about 0.6 g meal/L (C/N ratio of 10). The data indicated that the consumption of glucose by both cultures was 100% with the use of blood meal as sole nitrogen source. However, the initial concentration of glucose needs to be one-fifth for full utilization of glucose by *A. niger* in comparison to that possible with the use of *T. harzianum*.

Efficacy with the use of cheaper carbon sources

The efficacies of the meals to serve as nitrogen sources with the use of cheaper carbon sources (C/N ratio of 10 with 2 g cassava meal or wheat

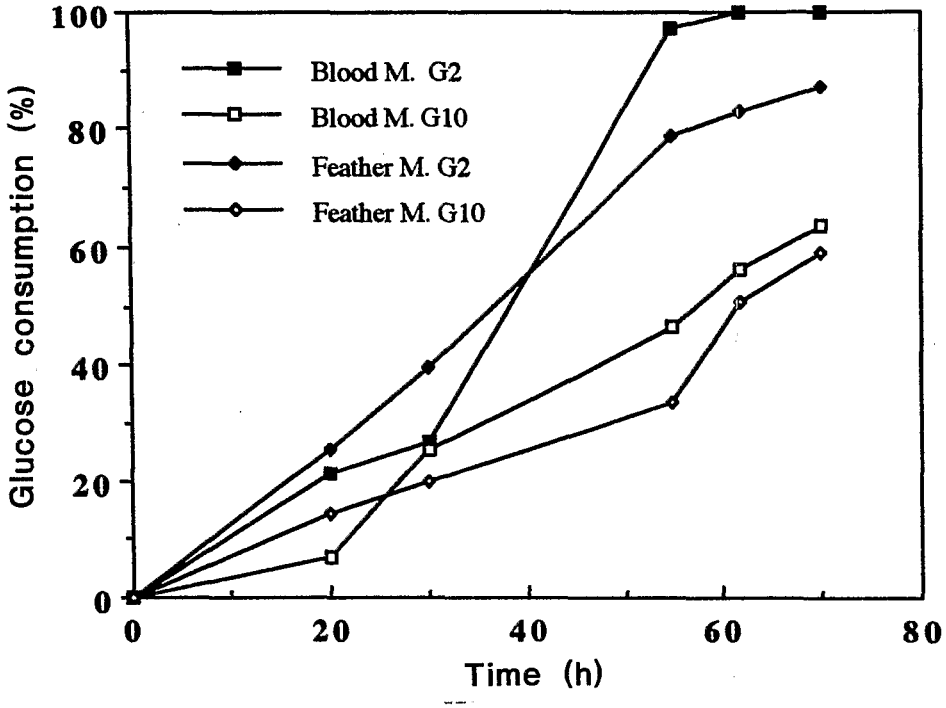


Fig. 3. Kinetics of glucose consumption by *Aspergillus niger* in 1.5 L fermenter.

bran/L) are shown in figures 5-6. The starch consumption by *A. niger* was higher than that by *T. harzianum* with the use of cassava meal as carbon source. In contrast, the consumption of starch + celluloses by *T. harzianum* was better than that by *A. niger* when wheat bran was used as sole carbon source, because this species is a strong cellulolytic fungus (Roussos and Raimbault, 1982; Roussos *et al.*, 1991b, 1991c).

Deodorization patterns

Patterns of deodorization of the meals were interesting, as evaluated qualitatively at the end of fermentation period. Complete deodorization of four different meals, used in the present studies individually as sole source of nitrogen, was possible with *T. harzianum* in the media containing glucose, wheat bran or cassava meal as carbon substrate. Similar was the case with *A. niger*, excepting the C/N ratio of 1 in medium containing feather meal and the C/N ratios of 1 and 7 in hair meal containing media. The data indicated that the deodorization of the meals is not due to their complete utilization as

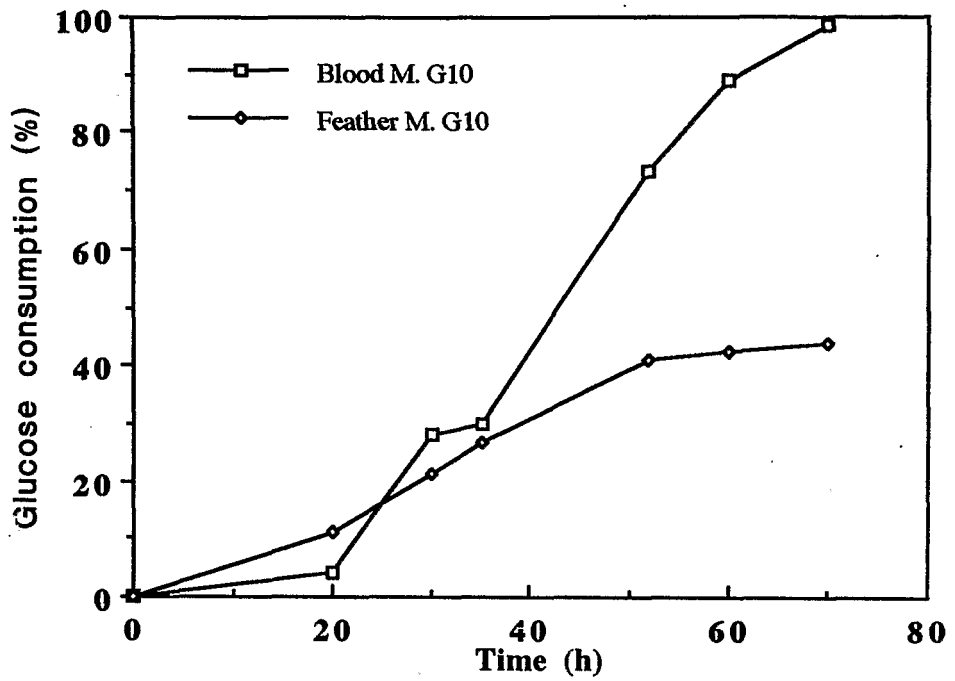
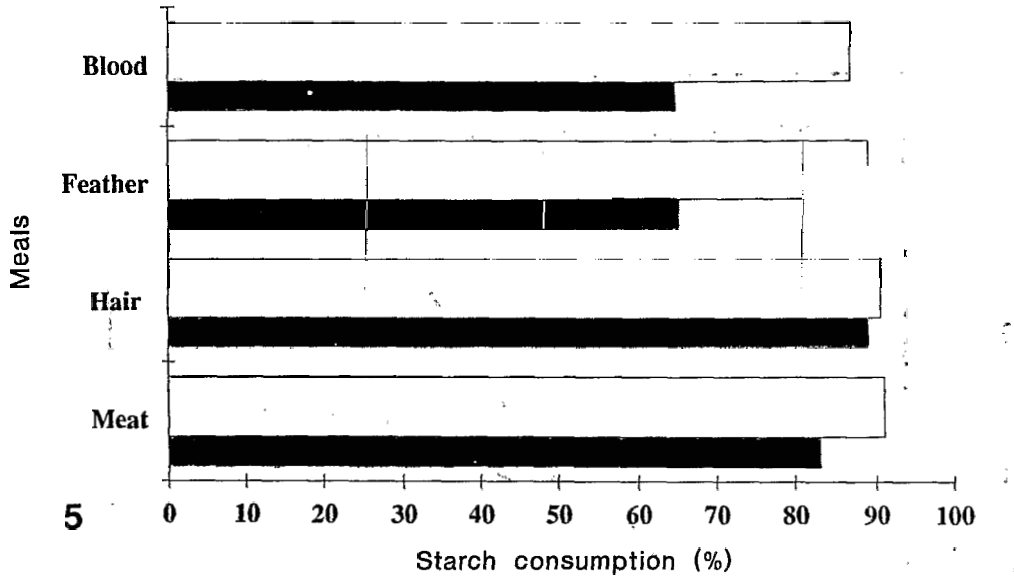


Fig. 4. Pattern of glucose consumption by *Trichoderma harzianum* against time in 1.5 L fermenter.

nitrogen source by the culture, because *A. niger* consumed about 75% of the total glucose at a C/N ratio of 1 in blood meal medium and still caused complete deodorization. In contrast, the odours were not eliminated by the culture in the media containing either feather or hairs meals in spite of 100% and 95% glucose consumption, respectively, at a C/N ratio of 1.

The deodorization of these meals from slaughter-house byproducts by *A. niger* and *T. harzianum*, or potentially any other microbial species, does not appear to have been reported so far. This opens up an entirely new avenue for microbial deodorization of these meals in the fermentation process, where the meal also serves as sole and cheaper nitrogen source. The offensive odour of the meals is a characteristic which limits their extensive and gainful utilization. The acceptability of the deodorized meals by animal feed manufactures and also by the animals will be much higher than what is existing now. Another possibility is to grow the desired microorganism on the meals



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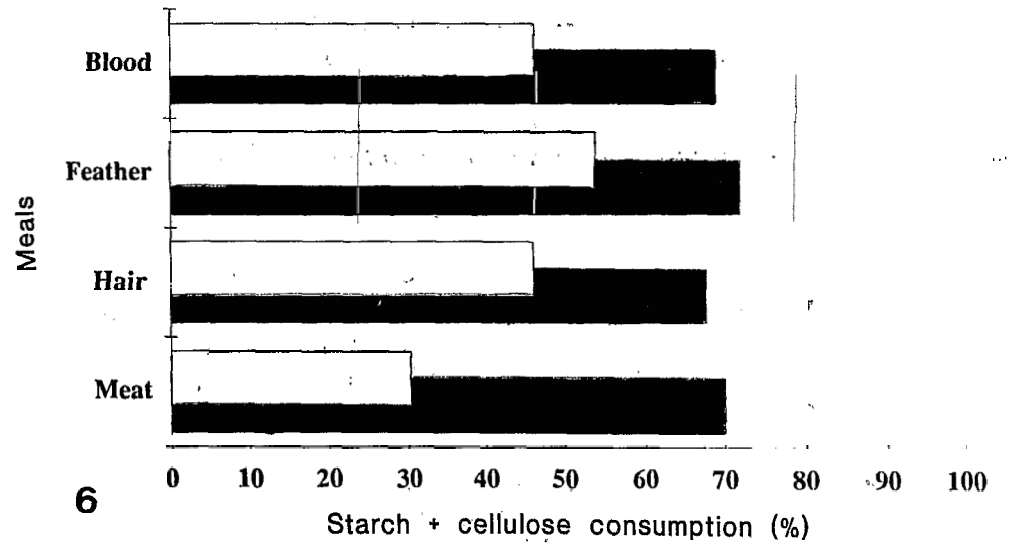

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Figs. 5-6.- 5: Efficacy of the meals as sole nitrogen sources in cassava meal containing medium for growth of *Aspergillus niger* (□) and *Trichoderma harzianum* (■). 6: Consumption of starch and cellulose by the cultures in media containing wheat bran and individual meals as carbon and nitrogen sources, respectively.

using lower C/N ratios and the resulting biomass, along with the non-utilized portion of the meal, can then be used as a component of animal feed. This would have distinct advantages, such as improved protein quality due to microbial biomass, elimination of offensive odours and enhanced acceptability. The use of these meals as organic nitrogen sources for mycelial growth also eliminates the need for the use of mineral nitrogen sources (Roussos *et al.*, 1991a), which are known to cause problems in the nutrition of monogastric animals (Ensiminger, 1978). In general, this may lead to better returns than what is possible at present.

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